

Listing of Claims

1. (Currently Amended) A method of diagnosing decreased vascular function in a subject that does not have symptomatic cardiovascular disease, comprising

selecting a subject that does not have symptomatic cardiovascular disease;

assaying the number of endothelial progenitor cells in a blood sample from the subject;

~~wherein the subject does not have symptomatic cardiovascular disease;~~

comparing the number of endothelial progenitor cells in the blood sample from the subject with a control, wherein the control is a number of endothelial progenitor cells in a blood sample from a control subject that does not have symptomatic cardiovascular disease and has a low Framingham Risk Score, and

identifying a subject with increased cardiovascular risk or decreased vascular function, wherein a decrease in the number of endothelial progenitor cells in the sample as compared to the[[a]] control indicates decreased vascular function in the subject.

2. (Currently Amended) The method of claim 1, wherein assaying the number of endothelial progenitor cells comprises

isolating the buffy coat from a blood sample of the subject;

isolating peripheral blood mononuclear cells from the buffy coat;

culturing the peripheral blood mononuclear cells ~~buffy coat~~ on a solid support coated with a first substrate, wherein cells from the peripheral blood mononuclear cells ~~buffy coat~~ adhere to the first substrate;

isolating non-adherent cells;

culturing the non-adherent cells on a solid support coated with a second substrate, wherein a subset of the non-adherent cells adhere to the second substrate and form colonies;

confirming that the subset of the non-adherent cells that adhered to the second substrate are endothelial progenitor cells by immunological assessment; and

counting the number of colonies on the solid support,

wherein the first substrate, or the second substrate, or both the first substrate and the second substrate comprise fibronectin, vitronectin, or collagen.

3. (Original) The method of claim 2, wherein a lower number of colonies on the solid support as compared to a control indicates decreased vascular function.

4. (Original) The method of claim 1, wherein assaying the number of endothelial progenitor cells comprises
determining the number of VEGFR²⁺CD31^{hi} cells in the sample.

5. (Original) The method of claim 1, wherein the control is a blood sample from a subject that does not have atherosclerosis.

6. (Original) The method of claim 1, wherein the control is a standard value.

7. (Original) The method of claim 2, wherein the first substrate comprises fibronectin.

8. (Original) The method of claim 2, wherein the first and the second substrate comprise fibronectin.

9. (Currently Amended) A method of diagnosing increased vascular function in a subject, comprising

selecting a subject that does not have symptomatic cardiovascular disease;

assaying the a number of endothelial progenitor cells in a first and second blood samples taken from the subject, wherein the second blood sample is taken from the subject after the first blood sample is taken from the subject;

~~wherein the subject does not have symptomatic cardiovascular disease;~~

comparing the number of endothelial progenitor cells in the first blood sample with the number of endothelial progenitor cells in the second blood sample, and

identifying a subject with increased vascular function, and wherein an increase in the number of endothelial progenitor cells in the second blood sample as compared to the first blood sample
~~control~~ indicates increased vascular function in the subject.

10. (Original) The method of claim 9, wherein the subject has been treated with a cholesterol-lowering agent.

11. (Currently Amended) The method of claim 10, wherein the ~~control~~ first blood sample is a blood sample taken from the subject prior to treatment with the cholesterol-lowering agent.

12. (Currently Amended) The method of claim 9, wherein assaying the number of endothelial progenitor cells comprises

isolating the buffy coat from a blood sample of the subject;

isolating peripheral blood mononuclear cells from the buffy coat;

culturing the peripheral blood mononuclear cells on a solid support coated with a first substrate, wherein cells from the peripheral blood mononuclear cells ~~buffy coat~~ adhere to the first substrate;

isolating non-adherent cells that do not adhere to the first substrate;

culturing the non-adherent cells on a solid support coated with a second substrate, wherein a subset of the non-adherent cells adhere to the second substrate and form colonies;

confirming that the subset of the non-adherent cells that adhered to the second substrate are endothelial progenitor cells; and

counting the number of colonies on the solid support,

wherein the first substrate, or the second substrate, or both the first substrate and the second substrate comprise fibronectin, vitronectin, or collagen.

13. (Original) The method of claim 12, wherein a higher number of colonies on the solid support as compared to a control indicates increased vascular function.

14. (Original) The method of claim 12, wherein the first substrate comprises fibronectin.

15. (Original) The method of claim 12, wherein the first substrate and the second substrate comprises fibronectin.

16. (Original) The method of claim 9, wherein assaying the number of endothelial progenitor cells comprises

determining the number of VEGFR²⁺CD31^{hi} cells in the sample.

17-19. (Canceled).

20. (Withdrawn) A method for screening for an agent that affects vascular function, comprising

administering a therapeutically effective amount of the agent to a subject, and

assessing the number of endothelial progenitor cells in a sample from the subject;

wherein an increased number of endothelial progenitor cells in the sample as compared to a control indicates that the agent affects vascular function.

21. (Withdrawn) The method of claim 20, wherein the subject is a non-human animal.

22. (Withdrawn) The method of claim 20, wherein the subject is a human.

23. (Withdrawn) The method of claim 20, wherein the agent is a cholesterol lowering agent.

24. (Withdrawn) The method of claim 20, wherein the control is the number of circulating endothelial cell in sample from a subject not administered the agent.

25. (Withdrawn) The method of claim 20, wherein the sample is a blood sample.

26. (Withdrawn) The method of claim 20, wherein the sample is a buffy coat sample.

27. (Withdrawn) The method of claim 20, wherein the endothelial progenitor cells are circulating endothelial progenitor cells.

28. (Withdrawn) The method of claim 20, wherein assaying the number of endothelial progenitor cells comprises

- isolating the buffy coat from a blood sample of the subject;
- culturing the buffy coat on a solid support coated with a first substrate;
- isolating the non-adherent cells;
- culturing the non-adherent cells on a solid support coated with a second substrate;
- enumerating the number of colonies on the solid support.

29. (Withdrawn) The method of claim 20, wherein assaying the number of endothelial progenitor cells comprises

- determining the number of VEGFR²⁺CD31^{hi} cells in the sample.

30-47. (Canceled).

48. (Currently Amended) A method of diagnosing increased cardiovascular risk or decreased vascular function in a subject, comprising

- selecting a subject that does not have symptomatic cardiovascular disease;

- assaying a number of senescent endothelial progenitor cells in a blood sample from the subject, wherein a senescent endothelial progenitor cell is a viable endothelial cell that exhibits clonal exhaustion in vitro;~~cannot divide, and~~

- comparing the number of senescent endothelial progenitor cells in the blood sample from the subject with a control, wherein the control is a number of senescent endothelial progenitor cells in a blood sample from a control subject that does not have symptomatic cardiovascular disease and has a low Framingham Risk Score, and

- identifying a subject with increased cardiovascular risk or decreased vascular function, wherein an increase in the number of senescent endothelial progenitor cells in the sample as compared to the[[a]] control indicates increased cardiovascular risk or decreased vascular function,~~and wherein the subject does not have symptomatic cardiovascular disease.~~

49. (Original) The method of claim 48, wherein the control is a standard value.

50. (Original) The method of claim 48, wherein the control is a number of senescent endothelial progenitor cells in a blood sample from a subject known not to be affected by a disease or disorder.

51. (Withdrawn) A method for screening for an agent of use in treating a cardiovascular disease, comprising
administering a therapeutically effective amount of the agent to a subject, and
assessing the number of senescent endothelial progenitor cells in a sample from the subject,
wherein a senescent endothelial cell is a viable endothelial cell that cannot divide;
wherein a decreased number of senescent endothelial progenitor cells in the sample as compared to a control indicates that the agent is of use in treating the cardiovascular disease.

52. (Withdrawn) The method of claim 51, wherein the control is a standard value.

53. (Withdrawn) The method of claim 51, wherein the control is a number of senescent endothelial progenitor cells in a blood sample from a subject known to be affected by a disease or disorder.

54. (Previously Presented) The method of claim 1, wherein vascular function comprises vascular contractility, brachial reactivity, atrial hyperplasia, or a combination thereof.

55. (New) The method of claim 1, wherein the control subject has a Framingham Risk Score of less than 1.5.

56. (New) The method of claim 2, wherein confirming that the subset of the non-adherent cells that adhere to the second substrate are endothelial progenitor cells by immunological assessment comprises contacting the cells with antibodies that specifically bind Vascular Endothelial Growth

Factor Receptor kinase insert domain receptor, contacting the cells with antibodies that specifically bind CD31, or measuring uptake of DiI-acetylated low density lipoprotein and co-staining with BS-1 Lectin.

57. (New) The method of claim 12, wherein confirming that the subset of the non-adherent cells that adhere to the second substrate are endothelial progenitor cells comprises immunostaining with antibodies that specifically bind Vascular Endothelial Growth Factor Receptor kinase insert domain receptor; contacting the cells with antibodies that specifically bind CD31; or measuring uptake of DiI-acetylated low density lipoprotein followed by co-staining with BS-1 Lectin.

58. (New) The method of claim 48, wherein the control subject has a Framingham Risk Score of less than 1.5.

59. (New) The method of claim 48, assaying a number of senescent endothelial progenitor cells comprises measuring endogenous beta-galactosidase.